REMARKS

Entry of the foregoing and reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested in light of the remarks which follow.

Applicants would first like to take the opportunity to thank Examiner Forman for meeting with Applicants' representative on December 2, 2004 and discussing the outstanding claims and issues discussed below.

As correctly noted in the Office Action Summary, claims 21-39 and 41-43 are pending. Claims 21 and 23-24 are amended herein. Basis for these amendments may be found throughout the specification and claims as-filed. Specifically, the amendments to claim 21 are supported by the specification as-filed. The term "without spatial resolution" is located at least at page 7, line 22 of the specification. The term "plurality" has been replaced with "population", which may be found for example at page 2, line 1. Claim 21 is further amended to recite that the population of single stranded DNA molecules are contacted with an array of hybridization probes, supported at least at page 2, line 1. The amendments reciting that the labels are mass labels and that the detection method is mass spectrometry are supported at least in claim 26 as-filed and on page 3 of the specification.

The claims are amended to recite that the recording of the quantity of each mass label takes place simultaneously. This Amendment is supported at least, for example, at page 15, paragraph 2, which states that when two templates have the same sequence in a particular cycle, the quantity of a particular label will be the sum of the quantities of the two sequences that share this sequence. This situation is only possible if the labels generated by the templates are measured at the same

time and occur in unique quantities to each other. The phrase "to ligate the double stranded portion...thereby forming an extended double stranded portion" in claim 21 is supported at least by claim 21 as-filed. Claims 23-24 are amended herein to recite "the heterogeneous population of single stranded DNA molecules", as supported at least by claim 21 as-filed. Thus, no new matter is presented by way of the present Amendment.

Claims 26, 33-39 and 41-43 are deleted herein without prejudice or disclaimer thereto. Applicants reserve the right to file at least one continuation application directed to any subject matter canceled by way of the present Amendment.

Abstract

As requested by the Examiner, an Abstract of the Disclosure set forth on a separate sheet is attached hereto. The Abstract is derived from the Abstract of the International PCT Application. Thus, no prohibited new matter is believed to be introduced.

Specification and Sequence Listing

The specification is amended herein to recite the heading "Brief Description of the Drawings". A Sequence Listing pursuant to 37 C.F.R. §§ 1.821-825 is attached. The specification is amended herein to recite the proper sequence identifiers, as corresponding to the attached Sequence Listing.

Claim Rejections - 35 U.S.C. § 112, first paragraph

Claims 21-32 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

The Examiner argues that the specification does not provide disclosure supporting the phrases "without spatial separation" and "present in a unique amount". Applicants traverse. The subject matter of the claim need not be described literally (*i.e.*, using the same terms *in haec verba*) in order for the disclosure to satisfy the description requirement. M.P.E.P. § 2103.02. To this end, Applicants submit that these terms, as amended herein in claim 21, are supported by the specification.

Spatial separation

The term "without spatial separation" in claim 21 has been replaced herein by the term "without spatial resolution" as suggested by the Examiner. Clear support for this phrase may be found at least on page 7, line 22, which recites "a method for analyzing heterogeneous sub-populations of the nucleic acids without spatially resolving them". It appears that the Examiner construed this passage to mean that detection takes place without spatial resolution. As clarified at the interview with the Examiner on December 2, 2004, Applicants note that the passage states that it is the nucleic acids themselves that are not spatially resolved. Accordingly, this passage of the specification supports a method of sequencing a heterogeneous population of single stranded DNAs without spatial resolution, as recited in amended claim 21. Therefore, Applicants submit that Claim 21 complies with the written description requirement.

Unique Amount

Applicants submit that the term "present in a unique amount" is not new matter and is supported in the specification. In response to the objection raised in items 3 and 4 of the Office Action, Applicants point out that the feature that the DNA templates are present in a unique amount is supported by the originally filed application at page 1, line 34. Applicants submit that the skilled artisan would construe the feature that the DNA templates are present in a unique amount to mean that each DNA molecule must be present in an amount different from every other DNA molecule. In particular, page 7, line 20, refers to the relative quantities of the DNA templates, suggesting that these must be present in different amount. Also, the specification at page 15, lines 20-25 states that the sequences being analyzed are present in different quantities. Therefore, Applicants argue that the application describes this feature of the invention in sufficient detail to make it available to the skilled artisan. However, as discussed in the interview with the Examiner, Applicants further submit that the correlation between the beginning amounts of each DNA molecule present in the reaction with the amount of mass label released at the end of each cycle of the reaction is also disclosed and supported in the specification.

Applicants turn to the method of the present invention in order to further clarify that "unique amount" and its link with the amount of tag released is supported by the specification, and again note that "unique amount" refers to the fact that each DNA molecule is present in a different amount from the other DNA molecules present in the reaction. By way of reference for the discussion below, Applicants again submit as Exhibit A, four diagrams showing the steps of the present invention ("Schmidt and Thompson 1-4").

The present invention, as recited in claim 21, is a method for sequencing a heterogeneous population of single stranded DNA, wherein the single stranded DNA *are not spatially resolved*. The beginning components of this method are:

- Single stranded DNA molecules *present in a unique amount*, each DNA molecule bearing a primer. The primer extends the single stranded DNA molecule providing a double stranded portion; and
- An array of hybridization probes. Each probe has a mass label cleavably linked to it.

The DNA molecules with their primers are brought into contact with the probes in the presence of ligase. The DNA molecule with primer ligates to the probe with the mass label, forming an extended double stranded portion.

The unligated probes are washed away and the mass label is cleaved. The quantity of the mass label is simultaneously recorded using mass spectrometry. By cleaving the mass label, it further frees the 3'-hydroxy end of the probe to undergo the ligation reaction again.

These steps are then repeated for a sufficient number of times to determine the sequence of the DNA.

Applicants submit that the above steps could not occur unless the DNA molecules present at the beginning of the reaction were not present in unique amounts, *i.e.*, amounts different from all of the other DNA molecules in the reaction. This is the only way to determine which sequence is involved in the reaction. This is because the reaction must be iterated until the entire nucleic acid has been sequenced, and the sequencing method of the present invention cannot be resolved in a single cycle, as set forth in step (f) of claim 21.

For example, Applicants refer to the second paragraph of page 15 of the specification. If one DNA molecule is present in amount X, and another DNA molecule is present in amount Y, then X + Y=the total amount of n-mer probe. Specifically, if two sequences of DNA template molecule in the claimed reaction require the same n-mer at the same time, this can be resolved using the claimed method. The *quantity* of a particular n-mer in a particular ligation reaction will be the sum of the quantities of the two sequences that share this n-mer at the same location. Once cycle of the present inventions (*i.e.*, steps (a) through (e) of claim 21) can be compared with previous and subsequent cycles to identify such sums. If one sequence is AGTC in amount X and another is AGGG in amount Y, the frequency of AG is X+Y, and frequency may be determined using the claimed method. It would be impossible to track which n-mer goes with which DNA molecule being sequenced unless the DNA molecules are present in different amounts.

The sequence of the nucleic acid at issue is not resolved in one reaction, but rather is resolved after sufficient amount of ligation cycles are run so that the sequence can be resolved quantitatively over all of the reactions. By definition, the DNA molecule templates are present in different (unique) amounts from each other or else the above, as recited in the specification, would not be possible. Applicants submit that sufficient support and explanation for the unique amount of DNA molecules, and the relationship between the unique amount and the amount of label seen at the end of each ligation cycle is clear form the specification as-filed.

In light of the above remarks, Applicants request that the rejection under 35 U.S.C. § 112, first paragraph be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 21-39 and 41-43 stand rejected under 35 U.S.C. § 112, second paragraph as purportedly indefinite.

Claims 21-32 stand rejected for the recitation "the plurality of single stranded DNA molecules" because it is purportedly unclear whether the recitation refers to the "heterogeneous population" of line 1 or another DNA molecule. As recommended by the Examiner, the term "plurality" is replaced herein with "population". The term "population" is recited earlier in the claim, and so Applicants submit the term "population" would clear and has appropriate antecedent basis.

Claims 21-32 stand rejected for the recitation "the array containing all possible base sequences being incapable of ligation to each other" because this phrase purportedly lacks proper antecedent basis. Part (a) of claim 21 has been amended herein to provide antecedent basis for the term "array". To this end, Applicants note that the probes are hybridized to the heterogeneous population of single stranded DNA molecules.

Claims 21-32 stand rejected for the recitation "to ligate the double stranded portion of each DNA molecule, the probe bearing the base thereby to form an extended double stranded portion" because it is purportedly unclear what is ligated to form the extended portion. Part (a) of claim 21 is amended herein to clarify that the probe is ligated to the double stranded portion of the DNA molecule to form the extended portion.

Claims 21-32 stand rejected for the recitation of "sequencing...DNA molecules simultaneously", as the claim purportedly does not recite a method step of simultaneous detection. In addition, the Office Action states that the last step

requires repetition of the steps a-e (*i.e.* contacting, removing, cleaving, recording and activating) to determine the sequence of each molecule. Applicants submit that it is clear that each DNA molecule in the population is sequenced simultaneously, but that not that all steps in the sequencing method take place simultaneously. Any other construction of this claim is inconsistent with the present invention, as the sequencing method is an iterative method. To this end, part (d) of claim 21 has been amended herein to specify that the mass labels generated in each cycle are detected simultaneously by mass spectrometry.

Claims 21-32 stand rejected for the recitation of "the ligated probes" because the recitation purportedly lacks proper antecedent basis in claim 21. This term is removed from the claims herein, and thus this rejection is moot.

Claims 21-32 stand rejected for the recitation of "the quantity" because the recitation purportedly lacks proper antecedent basis in the claim. Claim 21 is amended herein to clarify antecedent basis.

Claim 22 stands rejected because it is purportedly unclear how the "sub-arrays" differ from the array. Applicants submit that claim 22 clearly recites that the sub-arrays must together contain all the possible base sequences. Thus, it would be clear to the skilled artisan that a sub-array is a part of an array of hybridization probes. In support of this claim interpretation, Applicants refer to the last paragraph of page 2 of the specification as-filed. Further more, Applicants note that arrays containing probes having all possible nucleotides of a common length will contain probes that are complementary to one another. For example, an array of all possible 4-mers will contain the 4-mer AAAA and the complementary 4-mer TTTT. These 4-mers are capable of hybridization to one another. To avoid cross hybridization, it

would be preferable to use sub-arrays, each of which contain a portion of the 4-mers that are not complementary and to perform to or more sequential hybridizations and ligations.

Claim 23 stands rejected for the recitation "the target DNA population" because the recitation purportedly lacks proper antecedent basis in the "heterogeneous population" of Claim 21. Claim 24 stand rejected for the recitation "the initial DNA sample" because the recitation purportedly lacks proper antecedent basis in the "heterogeneous population" of Claim 21. Claims 23 and 24 have been amended herein to provide proper antecedent basis from claim 21.

Claims 26, 33-39 and 42-43 also stand rejected as purportedly indefinite. Claims 26, 33-39 and 42-43 are canceled herein, and thus this rejection is moot.

Claim Rejections - 35 U.S.C. § 102(b)

Claims 21-32 stand rejected under 35 U.S.C. 102(b) as being anticipated by Southern et al (WO 95/04160) ("Southern"). The Office Action states that the term "unique amount" is given its broadest reasonable interpretation consistent with the specification wherein it is not described and consistent with the claim wherein the uniqueness of the amount is not defined. Thus, the Examiner argues that the term the "unique amount" can be interpreted as each DNA molecule has a unique amount (*e.g.* one spot of vector sequence), but not different from the amount of other DNA molecules. Applicants traverse.

"[A]nticipation requires the presence in a single prior art disclosure of all elements of a claimed invention as arranged in the claims." *Jamesbury Corp. v.*

Litton Industrial Products, Inc., 225 U.S.P.Q. 253, 256 (Fed. Cir. 1985). Southern fails to describe or even suggest all of the elements of the rejected claims.

The present invention is directed to method for sequencing a heterogeneous population of single stranded DNA, wherein the single stranded DNA *are not spatially resolved*. The single stranded DNA molecules *present in a unique amount*, each DNA molecule bearing a primer. The primer extends the single stranded DNA molecule providing a double stranded portion; and an array of hybridization probes.

The claimed sequencing method is distinguished from the method disclosed in Southern because Southern fails to disclose the two requirements of the presently claimed invention that *each DNA molecule template is present in a unique amount* and that *the templates are not spatially resolved*. These differences result in the claimed sequencing method being able to sequence multiple DNA templates without spatial resolution or separation. This benefit eliminates the requirement to separate templates before sequencing, and enables mixtures of templates to be sequenced.

Southern fails to recite that the DNA templates are not spatially resolved and in fact Southern requires that the templates be spatially resolved

In contrast, the method of Southern *requires spatial resolution* of the templates. In fact, the method of Southern cannot sequence multiple templates without spatial resolution because the method of Southern has no way of assigning particular mass labels (and therefore sequences) to particular templates. Amended claim 21 is directed to a method of sequencing a heterogeneous population of single stranded DNA molecules without spatial resolution. As noted earlier in this Response, the phrase "without spatial resolution" refers to the DNA molecules.

Thus, the DNA template molecules of the presently claimed invention may not be spatially resolved or separated during any step of the sequencing method.

The Examiner states that Southern *et al.* teaches methods of simultaneously sequencing a plurality of different sequences. However, Applicants note that the methods of Southern utilize spatially resolved DNA templates. In one embodiment, separate DNA templates are immobilized on individual pins (*see* page 17, lines 9-11 of Southern). In the other embodiment disclosed, the DNA templates are spaced apart from one another on a support (see page 17, lines 27 to 31 of Southern). Therefore, the methods disclosed in Southern *et al.* do not anticipate the method of claim 21 because there is no disclosure of the feature that the DNA molecules are spatially resolved.

Southern fails to disclose that the DNA molecule templates are present in unique amounts

With regard to the requirement of the present invention that the DNA molecule templates are present in amounts unique from each other, Applicants refer to the comments above set forth for the rejection under 35 U.S.C. § 112, first paragraph. As stated, Applicants submit that it is clear from the specification that the term "unique amount" not only refers to the requirement that the amount of each DNA template molecule be present in an amount different from the other DNA template molecules in the heterogeneous population, but that the unique amount of DNA template relates to the end amount of label released in the reaction. The method of claim 21 requires each DNA molecule to be present in a unique amount. Claim 21 is novel over the disclosure of Southern, because Southern does not disclose

sequencing multiple DNA templates, each of which is present in an amount that is different from every other template.

By way of explanation, Applicants again submit as Exhibit A, pictures illustrating the differences between Southern and the present invention. Exhibit A as relating to Southern show the result of trying to sequence two different templates without spatial separation using the method of Southern. Southern 2 shows the two templates. Southern 3 shows the templates following ligation of the probe, and Southern 4 shows the templates after cleavage of the mass labels. The mass labels released are detected by mass spectrometry. *However, it is not possible for the method of Southern to assign the mass label to a particular template.*

The present invention overcomes this problem by having each DNA molecule present in a unique amount. The figures relating to the claimed method (Schmidt and Thompson) show that the quantity of the initial DNA molecule is related to the amount of label released. Schmidt and Thompson 4 shows that the amount of each mass label in the mass spectrum enables the mass labels to be assigned to a particular template.

In summary, the feature that the templates are not spatially resolved, and the feature that the templates are present in unique amounts are both required for sequencing of multiple templates. Either feature on its own does not enable the sequencing of multiple templates without spatial resolution. Accordingly, these features act synergistically. Neither of these elements, as claimed in the present invention, are recited or even suggested by Southern.

In light of the above remarks, Applicants submit that the rejection under 35 U.S.C. § 102 should be withdrawn.

Claim Rejections - 35 U.S.C. § 103(a)

Claims 32-39, 41-43 stand rejected under 35 U.S.C. 103(a) as purportedly unpatentable over Southern et al (WO 95/04160) and Drmanac (WO 95/09248) and the Stratagene Catalog (1989, page 39). Applicants note that the Examiner has rejected claim 32. However, in the Office Action on page 9, paragraph 10, the Examiner notes that Southern with Drmanac and the Stratagene Catalog combined are directed to kits and that claim 32 is directed to a kit. Applicants note that claim 32, as ultimately dependent on claim 21, is not directed to kits. Thus, Applicants address this rejection as pertaining to claims 33-39, which are directed to kits. Claims 33-39 and 41-43 are canceled herein, without prejudice or disclaimer thereto. Thus, the rejections under 35 U.S.C. § 103 are moot.

Double Patenting

Claims 1-32 are *provisionally* rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-18, 20-24, 27 and 28 of copending Application No. 09/462,408. The Examiner states that although the conflicting claims are not identical, they are not patentably distinct from each other. Claim 26 is canceled herein. With regard to the remaining claims, Applicants request that this rejection be placed into abeyance until allowable subject matter is determined.

CONCLUSION

From the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully requested to telephone the undersigned so that prosecution of the application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: December 7, 2004

By: _____
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EXHIBIT A

Southern 1 - The components

Single Template

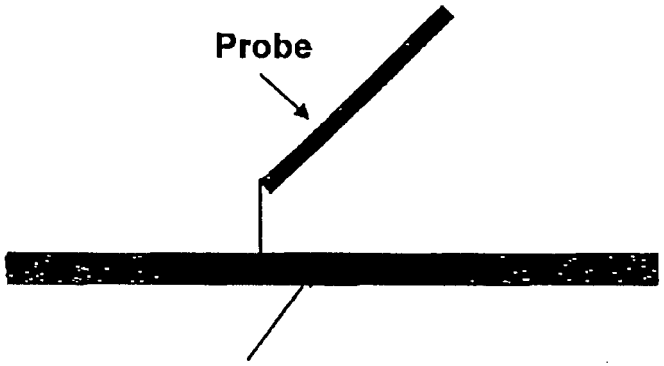


EXHIBIT A

Southern 2 – Hybridising a template to probe present in a reaction zone

Method disclosed in Southern

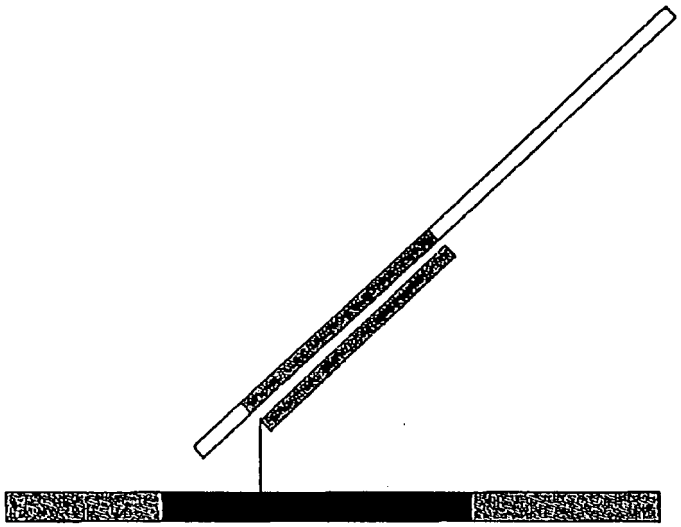


Diagram showing why the method of Southern does not permit sequencing of two templates in a single reaction zone

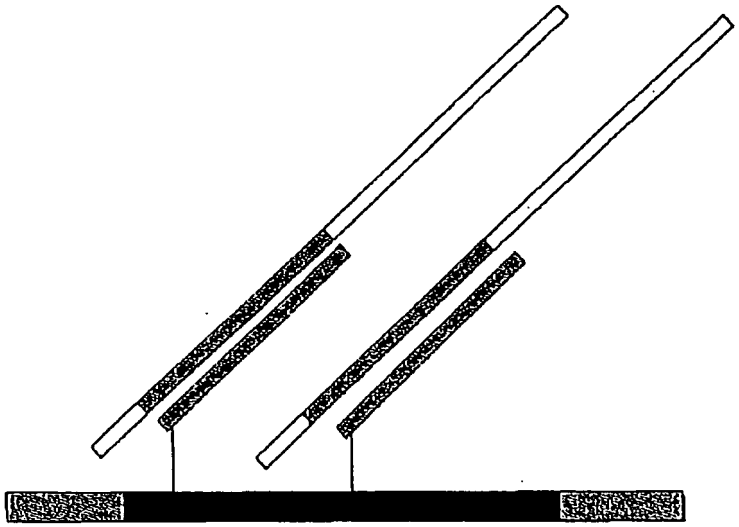


EXHIBIT A

Southern 3 - Ligating Mass Tagged Oligonucleotides

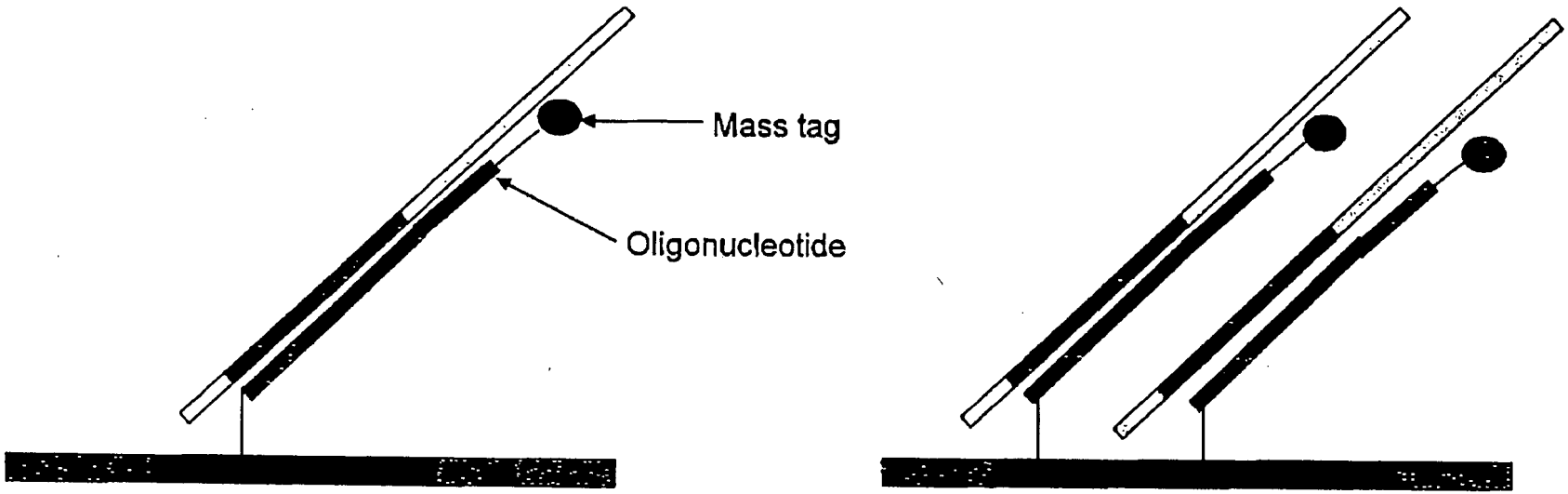
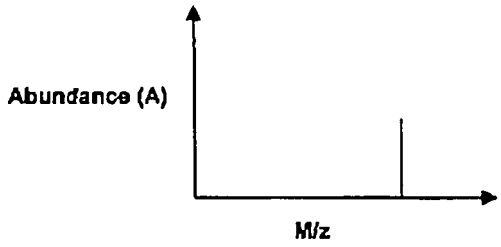
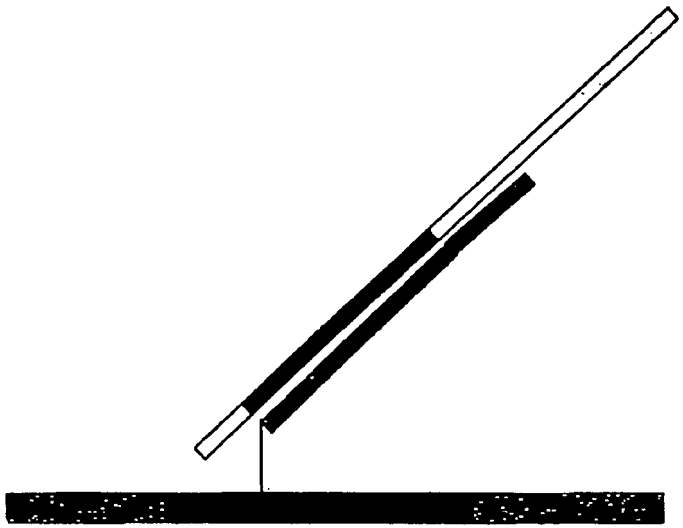


EXHIBIT A

Southern 4 - Cleave, Desorb and Detect Mass Tags

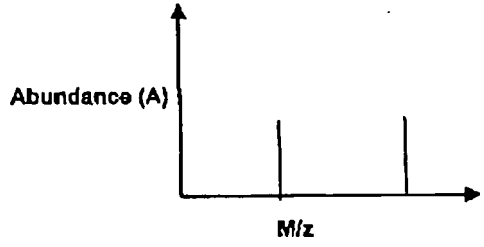
Mass spectrum showing mass and abundance of released mass tags

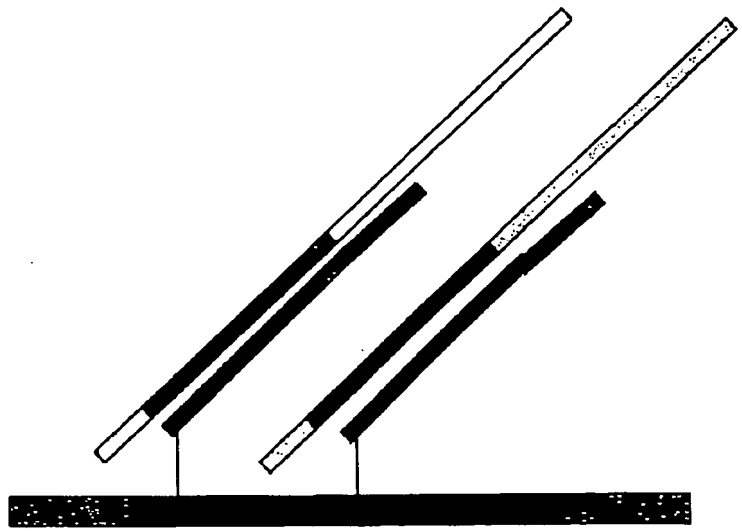




The mass tag detected is used to identify the sequence of the template adjacent to the region complementary to the probe

Mass spectrum showing mass and abundance of released mass tags

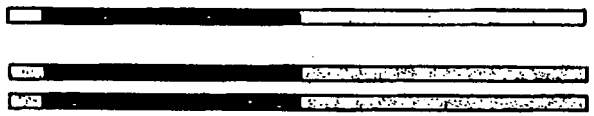




The mass tag detected cannot be assigned to a template and therefore, no sequence information can be obtained for either template

EXHIBIT A

Schmidt & Thompson 1 - The components



2 or more Templates in different quantities

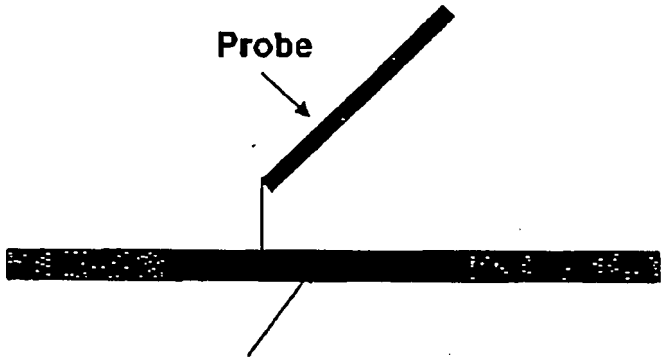


EXHIBIT A

Schmidt & Thompson 2 - Hybridising templates to a probe present in a reaction zone

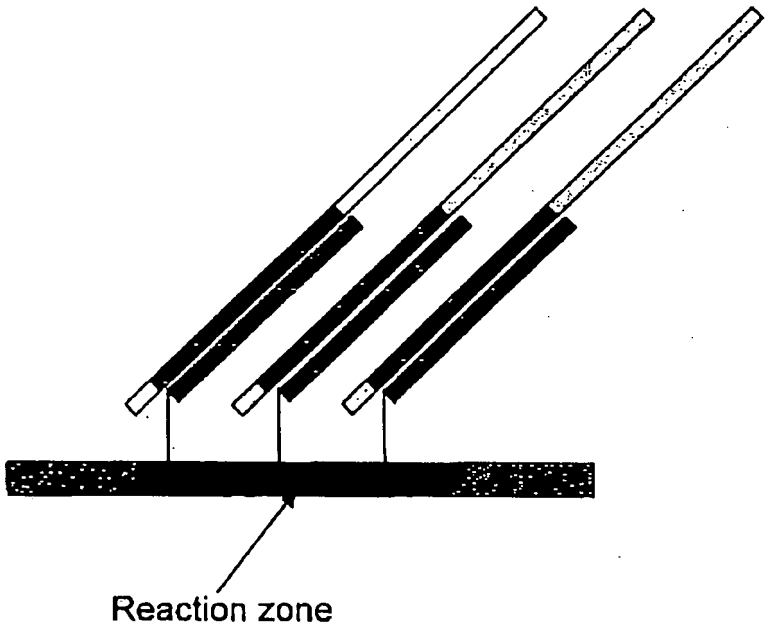


EXHIBIT A

Schmidt & Thompson 3 - Ligating Mass Tagged Oligonucleotides

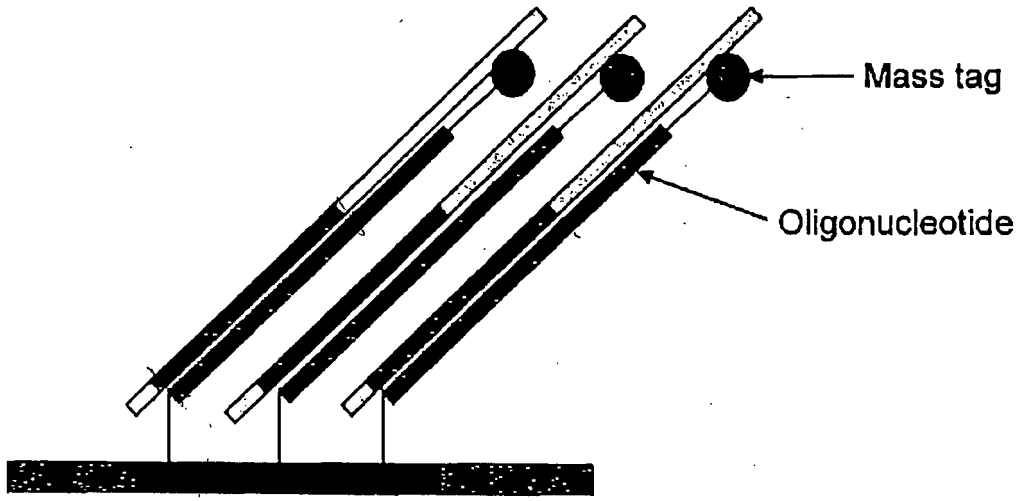
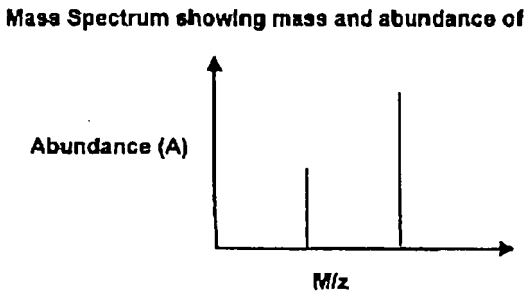
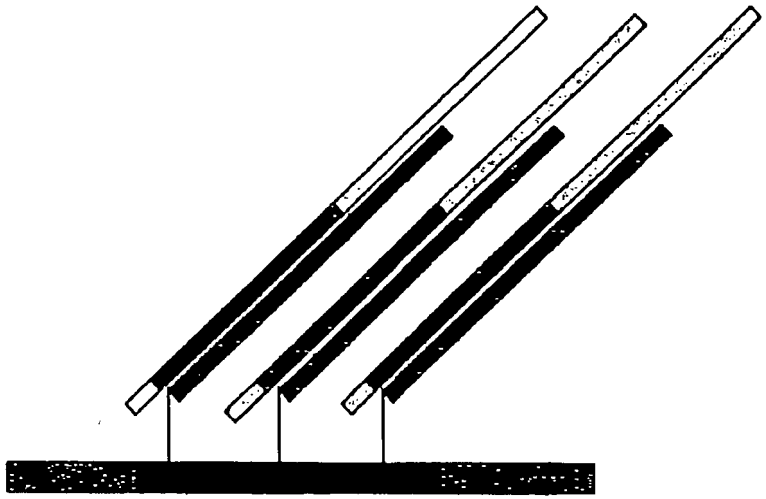


EXHIBIT A

Schmidt & Thompson 4 - Cleave and Detect Mass Tags





The abundance of each mass tag detected is used to identify the template to which it relates. Each mass tag is then used to identify the sequence of the corresponding template.